

Increase in arachidonic acid metabolites in the urine of female rats with cyclophosphamide-induced cystitis

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OBJECTIVES

Cyclophosphamide (CYP)-induced bladder inflammation is a well established pre-clinical model for interstitial cystitis. CYP treatment results in a local inflammation, bladder overactivity (OAB) and painful sensations. Bladder inflammation could excite the detrusor muscles with chemical mediators and activate the afferent nerve terminals. Mediators of the arachidonic acid cascade have been suggested to play a role in contributing to detrusor muscle contractility and modulating activity of bladder afferent nerves (1).

The present study aimed to:

- investigate the changes in urinary prostanoid mediators, particularly prostaglandin E2 (PGE2), leukotriene B4 (LTB4), thromboxane B2 (TXB2) and isoprostane, in the experimental model of CYP-induced cystitis in the female rat,
- correlate the levels of these mediators with the intensity of inflammatory response.

METHODS

- Twelve female Sprague Dawley rats (225-250 g) were used in the present study.
- Chemical cystitis was induced by a single intraperitoneal injection of CYP (150 mg/kg in saline, 10 mL/kg n=8). Four rats were used as control.
- Urine samples were collected using metabolic cages over an hour at different time points before and after CYP administration: -24 (control), +2, +6, +24, +48 and +72 hours.
- One hour before urine collection, rats received 2 mL of water by gavage to ensure enough urine volume for assays.
- Some rats were then sacrificed at -24 (control, n=4), +24 (n=4) and +72 hours (n=4), and urinary bladders removed.
- Different inflammatory parameters were assessed: whole urinary bladder wet weight, urinary bladder wall thickness, gross macroscopic analysis (oedema & haemorrhage using Gray's criteria (2), Figure 1F) and myeloperoxidase (MPO, index of granulocyte infiltration) activity.
- PGE2, LTB4, TXB2 and 15-isoprostane F2t were quantified using enzyme immunoassays (EIA) and normalised to total urine volume collected over an hour.

RESULTS

Acute CYP treatment induced alteration of the urinary bladder, oedema formation and granulocyte infiltration

- The administration of CYP (150 mg/kg i.p.) induced an increase in urinary bladder wet weight (p<0.05, Figure 1A) and wall thickness (Figure 1B) compared to control.
- Gross macroscopic analysis showed the presence of a strong oedema, a dilation of blood vessels, erythema and, in some animals, petechial haemorrhages (p<0.001 vs "control", p<0.01 vs "CYP +24h", Figures 1C & 1D).
- Compared to control, MPO activity was also increased (p<0.001) in all animals after CYP injection (Figure 1E).

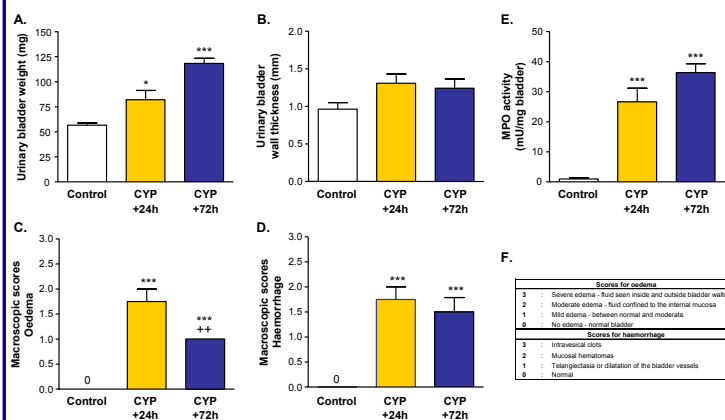


Figure 1. Alterations of urinary bladder parameters after acute CYP administration in female rats. (A) Urinary bladder wet weight, (B) urinary bladder wall thickness, (C) macroscopic score for oedema, (D) macroscopic score for haemorrhage, (E) myeloperoxidase activity, (F) macroscopic score description.

Each value represents mean \pm s.e.m. (n=4 for each time point). * p<0.05, *** p<0.001 different from « control » group, ++ p<0.01 different from « CYP +24 hours » group (one way ANOVA followed by Newman-Keuls multiple comparison test).

Increase in urinary prostanoid mediators after CYP-induced cystitis

- CYP treatment also resulted in a significant increase in urinary levels of PGE2, LTB4 and TXB2 compared to basal (-24 hours) (Figure 2).
- This increase was observed between 2 and 24 hours post-CYP administration, with a peak level at 6 hours (p<0.001, Figures 2A-C).
- 15-isoprostane F2t level was also significantly increased 6 and 72 hours post-CYP injection, compared to basal (p<0.05, Figure 2D).

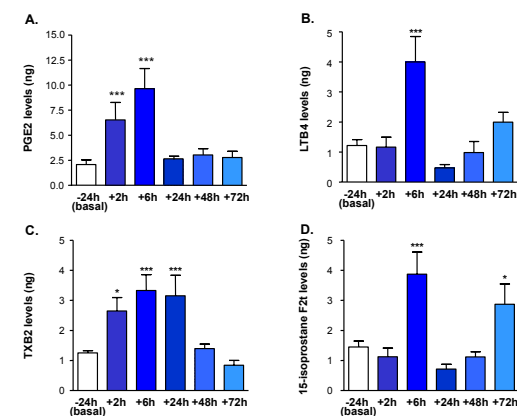


Figure 2. Arachidonic acid metabolite levels in whole urines collected over an hour of female rats after acute CYP administration. (A) PGE2, (B) LTB4, (C) TXB2 and (D) 15-isoprostane F2t.

Each value represents mean \pm s.e.m. (n=4-8). * p<0.05, *** p<0.001 different from « basal » value (one way ANOVA followed by Bonferroni/Dunnnett test).

DISCUSSION & CONCLUSIONS

In rats, a single intraperitoneal dose of CYP at 150 mg/kg is an experimental model for bladder hyperactivity characterized by an increase in micturition frequency and a decrease in bladder capacity (3). We have used this model to better assess urinary bladder inflammation. We found an increase in urinary bladder weight, macroscopic scores of oedema and haemorrhage, and MPO activity, signs of a moderate inflammation. These changes were observed 24 and 72 hours after CYP administration. We also observed an increase in urinary PGE2, LTB4, TXB2 and isoprostane, at early time point (6 hours) post-CYP injection. Their early release after CYP injection suggests that they are involved in the acute phase of inflammation. LTB4 synthesis shows the involvement of the lipoxygenase pathway, now of first interest to evaluate resolution of inflammation by the assessment of new markers such as lipoxins. Previous studies have also described an increase in urinary bladder COX-2 and PGE2 expressions, and urinary PGE2 (4) in the same experimental model. Interestingly, activation of TXB2 and PGE2 receptors induced contractions of the isolated urinary bladder from rats (5) and humans (6). In addition, an increase in urinary PGE2 was reported in patients with OAB (7) suggesting that PGE2 plays an important role in this pathology.

In conclusion, arachidonate metabolites are differentially regulated in the course of CYP-induced cystitis, and may play an important role in the genesis of urinary bladder inflammation and eventually in OAB observed in rats pre-treated with CYP.

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